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ORIGINAL PAPER

Shiori Hashimoto · Nicholas Chiorazzi
Peter K. Gregersen

The complete sequence of the human *CD79b* (Ig β /B29) gene: identification of a conserved exon/intron organization, immunoglobulin-like regulatory regions, and allelic polymorphism

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Abstract We determined the complete genomic sequence of the human *CD79b* (Ig β /B29) gene. The *CD79b* gene product is associated with the membrane immunoglobulin signaling complex which is composed of immunoglobulin (Ig) itself, associated in a noncovalent fashion with *CD79b* and a second polypeptide chain, *CD79a* (Ig α /mb1). The sequence and exon/intron organization of the human and mouse *CD79b* genes are highly similar. The gene organization suggests that some variant forms of *CD79b* may arise by virtue of alternative splicing of mRNA. In addition, a number of conserved regulatory sequences commonly found in *Ig* genes are present in sequences which flank the human *CD79b* gene. Some of these sequences are distinct from those found in the *CD79a* promoter. These differences may explain why transcription of *CD79b*, but not *CD79a*, is observed in plasma cells. A new *Taq* I restriction fragment length polymorphism is described that is not associated with any structural polymorphisms of the expressed *CD79b* polypeptide.

Introduction

The recognition of specific antigen by B lymphocytes depends on the function of the membrane immunoglobulin (mIg) receptor complex, a multimeric complex which, in addition to mIg, includes at least two associated glycoproteins designated *CD79a* (Ig α /mb1) and *CD79b* (Ig β /B29) (Reth 1992; Schlossman et al. 1994). These immunoglobulin(Ig)-associated molecules participate in signal transduction and contain highly conserved intracytoplasmic domains which appear to associate with intracellular signalling molecules. The *CD79b* molecule is of particular interest because it appears that several different forms of *CD79b* may exist (Friedrich et al. 1993; Hashimoto and co-workers, unpublished results), the molecular basis of which remains undefined. In addition, unlike *CD79a* (Ig α /mb1), the transcription of *CD79b* is maintained in mIg plasma cells (Hashimoto et al. 1993). In order to begin to understand these features of *CD79b*, we determined the complete sequence of the *CD79b* gene, and in the course of this work we also defined a new *Taq* I polymorphism.

Materials and methods

Isolation of cosmid clones

A chromosome 17-specific cosmid library was obtained from the Reference Library Data Base of the Imperial Cancer Research Fund (London, Great Britain). Filters #1 and #2 (Library number 105, set 12) were screened with a cDNA probe containing the full-length *CD79b* coding sequence, using standard conditions of high-stringency hybridization. Duplicate positive colonies were identified by position on the filter grid, and provided to us by the Reference Library.

DNA sequencing strategy

DNA sequencing was performed by using a *Taq* DyeDeoxy™ Terminator Cycle Sequencing Kit (ABI, Foster City, CA). Primers specific for portions of the *CD79b* gene were synthesized (Biosearch 8700; Millipore, Bedford, MA) as needed for sequence analysis by a primer walking strategy. Twenty-two primers were used to complete the se-

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number L27587

S. Hashimoto¹ · N. Chiorazzi · P. K. Gregersen (✉)
Department of Medicine, North Shore University Hospital,
350 Community Drive, Manhasset, NY 11030, USA

N. Chiorazzi · P. K. Gregersen
Department of Medicine, Cornell University Medical College,
New York, NY 10021, USA

¹ Present address: Department of Neurology, Tokyo Women's Medical College, 10 Kawada-cho, Shinjuku-ku, Tokyo, Japan

sequence analysis of approximately 3800 base pairs (bp). Sequencing reactions were analyzed on an Applied Biosystems (Foster City, CA) Automated Sequenator Model 373 A. The most 3' sequencing primer F13 (5' CGG ACA GGG GAA GTG AAG TG 3') was used to confirm the 3' extent of cosmid clones.

Southern blot analysis

Ten μ g of genomic DNA were digested overnight with *Taq* I (Gibco/BRL, Gaithersburg, MD), electrophoresed through 1% agarose gel, and transferred onto nylon membranes (Micron Separations, Westboro, MA). After cross-linking using the Stratalinker UV cross-linker (Stratagene, La Jolla, CA) for 90 s, the filters were prehybridized for 15 min at 60°C in QuikHyb solution (Stratagene). Filters then were exposed for 1 h at 60°C in QuikHyb solution to a 32 P-labeled, 800 bp probe derived from the Ig β cDNA, and subsequently washed twice for 15 min at room temperature in 2 \times standard sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), followed by a 30 min wash at 65°C in 0.1 \times SSC, 0.1% SDS. Filters were exposed overnight on Kodak XAR5 film at -70°C.

Results

Isolation of cosmid clones for *CD79b*

The *CD79b* gene has been localized to chromosome 17q23 (Wood et al. 1993). We therefore obtained a chromosome 17-specific cosmid library for screening from the Reference Library Data Base of the Imperial Cancer Research Fund. The cosmid library was screened with a cDNA clone for human *CD79b* which was previously isolated in this laboratory (Hashimoto et al. 1993). Seven positive clones were identified on the initial screening. These clones were provided to us by the ICRF laboratories and we subsequently identified two cosmids which appeared to contain the entire *CD79b* gene. These clones were identified by their ability to be amplified by PCR primers specific for the 5' end of the *CD79b* cDNA sequence (primer pairs F24/R5), and by successful priming of sequence reactions, using a primer (F13) specific for the 3' end of the cDNA sequence.

Sequence analysis of the *CD79b* gene

The entire genomic sequence of the *CD79b* gene was obtained and is shown in Figure 1. The overall intron/exon organization is highly similar to that reported for the mouse *CD79b* gene (Reth 1992). The *CD79b* genes of both human and mouse contain 6 exons. Exon 1 encodes a typical leader peptide sequence; this is followed by exon 2 which encodes a short peptide preceeding the Ig-like domain encoded by exon 3. Exon 4 encodes the hydrophobic transmembrane (TM) portion of the *CD79b* molecule, whereas the coding regions for the intracytoplasmic (CP) domains are divided between exons 5 and 6. As noted previously (Hashimoto et al. 1993), the mouse and human sequences are highly similar in sequence, especially in the TM and CP regions (>95% similarity in predicted amino acid sequence), as well as sharing the conserved exon/intron structure shown

h GACCACCGGTGGGGTAAGCACAGACAGAGGGGAGCA -305
m AGATGA.CCAGAGTGGGA..G..GG..TTA.A.A..

h CAGGCTTCCCCAGAAAGACTGAGAGGCC CCCAGAGGCATCCAGAGGACCCC -244
m .T...GT...T...T.C..GA..AACCCCTT...G..GTCT.T..AGAAC..AA

"E2-like"

h AGCTGTCTGCCCAAGCTGG GCGACCGCAACCTTAGCGGCCAGCTGACAAAAGCCTG -183
mG..A...G..A..AC.AC..TT.G.GT.C...A..A.....TG.....

"E3-like"

h CCGTCCCCCAGGGTCCCGGAGAGCTGGTGCCCTCCCTGGGTCCCAATTGTCATGGCAGG -122
mAT.....T...G..A.....T.....

uB/LyF1 SPI

h AAGGGGCTGTGTGAGGAAGAGCGGGGAGGACAGGCTGACAGCGGTGCAGTTACAGCT - 61
mAA..G.....A.....A.....CAG..T..AC..T..AG

h TTCTCTCAAGGAGCGCTCGGACGTGTGTACCGGTTTGGGGTCGGGACAGAGCGGTGACCATTG
m ..A.A.GTTTC..CC..CA ..A..CTCC.....C..A..A.....A.....

Fig. 2 Sequence comparison of the 5' UT regions of the human and mouse (Hermanson et al. 1988) *CD79b* genes. Conserved regulatory sequences are indicated

here. A 3' untranslated (UT) region of 461 bp separates the stop codon from the polyadenylation signal AATAAA.

In addition to interspecies similarities in the coding region and gene organization, there are also conserved *cis*-acting regulatory elements which are present in the flanking sequence of human *CD79b*. A comparison of the human and mouse 5' promoter region is shown in Figure 2. This region contains sequences which are known to be involved in the regulation of *Ig* genes. Among these are the highly conserved octamer sequence, as well as an ets binding site and an SP1 target sequence. A uB/LyF1 binding region was identified in the mouse and is partially conserved in the human sequence. An additional enhancer (E3-like) element was tentatively identified in the previously reported mouse sequence, and is present in a slightly altered form in the human *CD79b* promoter. An E2-like sequence is also found in the human sequence, but was not observed in the mouse (Hermanson et al. 1989). Both the human and mouse promoter sequences lack a 5' TATA box. Consistent with this, the mouse and human *CD79b* transcripts have been shown to initiate at multiple sites downstream of the octamer sequence (Hermanson et al. 1989; Omori and Wall 1993).

Enhancer elements can also be found in the 3' end of *Ig* genes (Staudt and Lenardo 1991), and for this reason we searched the 3' region of human *CD79b* for such sequences. We identified four potential sites of E elements, all of which had 7/8 nucleotides identical to the previously described E elements (Staudt and Lenardo 1991). These are indicated in Figure 1.

Identification of RFLPs in the human *CD79b* gene

We identified an allelic polymorphism associated with the *CD79b* gene after digestion with *Taq* I. As shown in Figure 3, two bands are observed in homozygous individuals. One band at 2.2 kilobases (kb) is constant in all individuals. A second band reflects a *Taq* I polymorphism which is biallelic in the population, with either a 1.8 kb or 2.5 kb

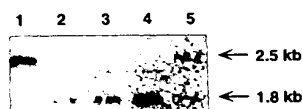


Fig. 3 Southern blot analysis of *Taq* I-digested genomic DNA from five unrelated individuals. A full-length *CD79b* cDNA probe was used. Two alleles have been observed in the population. Lane 1 shows an individual homozygous for the 2.5 kb allele, while lanes 2-4 show individuals heterozygous for the 1.8 kb allele. The individual in lane 5 is homozygous. All subjects contain a constant, and relatively weak, band at 2.2 kb

Table 1 Distribution of Ig β *Taq* I RFLPs in various ethnic groups.

	Caucasian (n = 20)	Black (n = 20)	Oriental (n = 20)	Total (n = 60)
2.5/2.5*	3	1	4	8
2.5/1.8	8	3	9	20
1.8/1.8	9	16	7	32

An asterisk indicates that Southern blot analysis of *Taq* I-digested genomic DNA, using a full-length *CD79b* cDNA probe, identified two polymorphic fragments of 2.5 kb and 1.8 kb

band present. Heterozygotes (lane 5, Figure 3) contain both of these bands, as well as the constant 2.2 kb band.

We analyzed 60 unrelated individuals for the presence of the *Taq* I RFLP. Subjects from various ethnic groups were examined, and Table 1 shows the results of this study broken down by ethnic group. All three ethnic populations appear to be in Hardy Weinberg equilibrium (null hypothesis not rejected by goodness of fit test).

Discussion

In this study we determined the complete sequence of the human *CD79b* (Ig β /B29) gene, and the sequence reveals a remarkable degree of similarity between the mouse and human homologues. These similarities relate to both sequence conservation within the coding region, as well as preservation of the overall exon/intron organization. The gene organization is of some interest for understanding the mechanisms by which variant forms of the *CD79b* molecule may be produced. It has recently been reported that a truncated form of mouse *CD79b* protein exists in which the C terminal portion of the cytoplasmic tail is deleted (Friedrich et al. 1993). The exact extent of this deletion has not been determined, nor has this been reported as yet in human *CD79b*.

Nevertheless, the exon/intron structure of the gene suggests that a possible mechanism for this deletion might be alternative splicing at the 3' end of the gene, such that exon 6 is removed from some transcripts. In addition, using an RNase protection assay, we observed truncated transcripts of the human *CD79b* in which a large segment of the extracellular portion of the molecule had been deleted (unpublished results). Interestingly, this deletion corresponds exactly to the removal of exon 3, again suggesting

alternative splicing as a mechanism for this variant transcript. The functional significance of these variant forms of *CD79b* has not yet been established.

A second interesting feature of the Ig-associated signaling molecules is their distinct pattern of transcriptional regulation. The *CD79b* gene is transcriptionally active throughout all stages of B-cell differentiation (Hermanson et al. 1989). Thus, *CD79b* is expressed with approximately the same timing as the onset of Ig heavy chain rearrangement, an event associated with Ig gene transcription (Blackwell et al. 1986). Consistent with this pattern of expression, the *CD79b* gene contains regulatory elements commonly found in Ig enhancers. Some of these elements, such as the octamer motif, are identical in the mouse and human *CD79b* sequences (see Figure 2), as well as in the Ig genes (Staudt and Lenardo 1991). This sequence is known to form a binding site for transcription factors Oct1 and Oct2. Other putative enhancer elements also exhibit similarity to the previously defined Ig enhancer sequences. Among these are the E3-like and E2-like sequences present in the human *CD79b* promoter region. It has recently been shown that the E3-like sequence in mouse Ig β binds nuclear extracts in gel shift assays (Omori and Wall 1993). In addition to these 5' enhancer elements, we have also identified candidate enhancer sequences in the 3' end of the gene, similar to the location of such enhancers in Ig genes. Four such regions are shown in Figure 1, each of which matches the published Ig enhancer elements in 7/8 nucleotides. Further studies will be required to determine whether these putative regulatory motifs are actually involved in the regulation of *CD79b* transcription.

As described previously, the major function of the *CD79b* gene product is in the formation of a membrane signalling complex associated with Ig at the surface of B cells (Reth 1992). At a minimum, this complex consists of Ig itself associated in a noncovalent fashion with *CD79b* and a second polypeptide chain, *CD79a* (Ig α /mb1). As we have reported previously, the *CD79b* gene is transcribed at high levels in plasma cells (Hashimoto et al. 1993). In contrast, the *CD79a* gene is not transcribed in plasma cells (Ha et al. 1992), consistent with the absence of surface Ig in these cells. The *CD79a* gene contains a promoter region which bears partial similarity to that seen in *CD79b*; both the ets and SP1 binding sites are present in the *CD79a* promoter, which also lacks a TATA box. However, no E3-like element is present in *CD79a*. In addition, a putative *cis*-acting sequence has been recently identified in *CD79a* which is the target for a novel DNA binding protein, BlyF (Feldhaus et al. 1992). BlyF is not present in plasma cells, and this may partially account for the lack of transcription of *CD79a* in plasma cells. Interestingly, a BlyF binding motif is not present in the *CD79b* 5' promoter region, again emphasizing the distinct patterns of regulation for these two genes. Given the absence of membrane Ig in plasma cells, it is at present unclear what function *CD79b* has at this stage of B-cell differentiation.

Wood and co-workers (1993) have recently shown that the human *CD79b* gene maps specifically to 17q23. This is of interest in view of the fact that translocations involving

17q23 have been observed in some cases of lymphocytic leukemia (Mitelman et al. 1990). It is tempting to speculate that the presence of numerous enhancer elements for B-cell transcription within the *CD79b* gene might enhance the expression of oncogenes translocated into this region. However, to our knowledge, a detailed molecular analysis of these translocations has not been performed.

Finally, we identified a new *Taq* I polymorphism of the *CD79b* gene. As shown in Table 1, a preliminary analysis of gene frequencies within various ethnic groups shows a relative preponderance of the 1.8 kb *Taq* I allele with an overall heterozygosity (H) of 0.33; the distribution of genotypes is consistent with Hardy-Weinberg equilibrium in all three ethnic groups. We did not detect any coding region polymorphisms associated with this *Taq* I RFLP, although we have not yet searched for other allelic polymorphisms in the flanking regulatory regions.

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References

- Blackwell, T. K., Moore, M. W., Yancopoulos, G. D., Suh, H., Lutzker, S., Selsing, E., and Alt, F. W. Recombination between immunoglobulin variable region gene segments is enhanced by transcription. *Nature* 324: 585-589, 1986
- Feldhaus, A. L., Mbangkollo, D., Arvin K. L., Klug, C. A. and Singh, H. BlyF, a novel cell-type- and stage-specific regulator of the B-lymphocyte gene mb-1. *Mol Cell Biol* 12: 1126-1133, 1992
- Friedrich, R. J., Campbell, K. S., and Cambier, J. C. The γ subunit of the B cell antigen-receptor complex is a C-terminally truncated product of the B29 gene. *J Immunol* 150: 2814-2822, 1993
- Ha, H., Kubagawa, H., and Burrows, P. D. Molecular cloning and expression pattern of a human gene homologous to the murine mb-1 gene. *J Immunol* 148: 1526-1531, 1992
- Hashimoto, S., Gregersen, P. K., and Chiorazzi, N. The human Ig- β cDNA sequence, a homologue of murine B29, is identical in B cell and plasma cell lines producing all the human Ig isotypes. *J Immunol* 150: 491-498, 1993
- Hermanson, G. G., Eisenberg, D., Kincade, P. W., and Wall, R. B29: A member of the immunoglobulin gene superfamily exclusively expressed on B lineage cells. *Proc Natl Acad Sci USA* 85: 6890-6893, 1988
- Hermanson, G. G., Briskin, M., Sigman, D., and Wall, R. Immunoglobulin enhancer and promoter motifs 5' of the B29 B-cell-specific gene. *Proc Natl Acad Sci USA* 86: 7341-7345, 1989
- Mitelman, F., Kaneko, Y., and Trent, J. M. Report of the committee on chromosome changes in neoplasia. *Cytogenet Cell Genet* 55: 358-386, 1990
- Omori, S. A. and Wall, R. Multiple motifs regulate the B-cell-specific promoter of the B29 gene. *Proc Natl Acad Sci USA* 90: 11723-11727, 1993
- Reth, M. Antigen receptors on B lymphocytes. *Annu Rev Immunol* 10: 97-121, 1992
- Schlossman, S. F., Boumsell, L., Gilks, W., Harlan, J. M., Kishimoto, T., Morimoto, C., Ritz, J., Shaw, S., Silverstein, R. L., Springer, T. A., Tedder, T. F., and Todd, R. F. CD antigens 1993. *J Immunol* 152: 1-2, 1994
- Staudt, L., and Lenardo, M. Immunoglobulin gene transcription. *Annu Rev Immunol* 9: 373-398, 1991
- Wood, W. J., Jr., Thompson, A. A., Korenberg, J., Chen, X.-N., May, W., Wall, R., and Denny, C. T. Isolation and chromosomal mapping of the human immunoglobulin-associated B29 gene (IGB). *Genomics* 16: 187-192, 1993